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The overall goal of our ONR-sponsored research is to pursue realistic molecular modeling strudies pertinnent to the related properties of protein stability, dynamics, structure, function, and folding in aqueous solution. We have developed mixed solvent systems to investigate the conformational behavior of peptides and proteins in response to a change in environment. We have also been studying hydrophobic hydration and how it may affect the diffusivity of molecules and how it may be harnassed in the design of biomaterials.

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FINAL REPORT

Grant #: N00014-95-1-484

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OBJECTIVE: To perform realistic molecular modeling studies pertinent to the related properties of protein stability, dynamics, structure, function, and folding in aqueous solution.

APPROACH: Mixed organic/water solvent systems are being developed for use in molecular dynamics simulations to better mimic experimental conditions. By comparing the detailed solvent-protein interactions occurring in pure water versus an aqueous organic solution, the role of the solvent in determining the stability of a protein in various conformations (e.g. native, partially unfolded and fully unfolded) should be evident.

ACCOMPLISHMENTS: The use of co-solvents with water in the study of protein behavior is so commonplace as to be routinely assumed. Despite this, the number of classical molecular dynamics simulations of protein systems which use co-solvents is still quite small in comparison to the large number of studies using pure water as solvent. The primary use of co-solvents is to provide a stabilizing or destabilizing environment for various components of protein structure in order to emphasize these characteristics for experimental monitoring.

We have developed co-solvent models of urea, guanidinium hydrochloride, dimethyl formamide (in addition to numerous alcohols). The structural and dynamic properties of the resulting aqueous solutions were compared with experimental observation. The models provide accurate representations of these solvents over a range of temperatures and concentrations. We are now applying these solvents to the study of how they affect protein conformation. Here we are using native, partially unfolded and completely unfolded structures of ubiquitin and comparing the conformational properties in 6M urea, 60% methanol and pure water. The simulations provide a

molecular-level description of the action of denaturants, which involves both direct effects on the protein (for example, disruption of hydrogen bonds) and indirect effects via changes in solvation upon the introduction of denaturant.

We have just completed a study of the effects of crocetin on oxygen diffusion in collaboration with Dr. J. Gainer. This project was suggested by Dr. H. Bright and we were challenged to provide a molecular model for the experimentally observed behavior. This area is of interest because the limitations of oxygen diffusion through plasma have been related to the onset of atherosclerosis and correlated with other factors associated with hypoxia, such as aging, smoking, hypertension, diabetes, and hypercholesterolemia. Additionally, the ability to increase the oxygen diffusion coefficient could prove to be of considerable utility to limit the consequences of hemorrhagic shock.

To this end a model for the diffusion process in dilute solutions has been developed by Gainer and co-workers in which diffusion is envisioned as the 'jumping' of solute from one cavity in solution to another, with an associated activation energy. The model predicts that addition of a hydrophobic co-solute with a positive volume of mixing should increase the spacing within the solution sufficiently to allow greater diffusion of oxygen. This is borne out by experiments in Gainer's lab.

Our molecular dynamics simulations show that the diffusion rate of oxygen through water is enhanced in the presence of crocetin. The mobility of oxygen through water is greater within a 5 Å layer around crocetin. This layer corresponds to the region of water whose structure is altered by the presence of the largely hydrophobic co-solute. The difference in the structure of water eases the passage of oxygen through the water 'lattice' by slightly increasing the average oxygen-oxygen interatomic distance within this layer. This coincides nicely with the predictions made by the diffusion model about the consequences of the change in molar volume upon addition of crocetin to water.

SIGNIFICANCE: These simulations provide a heretofore unavailable microscopic view of the effect of different solvents on the protein unfolding process and the various conformational states populated, as well as other systems of interest. Such studies are prerequisites for investigation of functional characteristics in organic solvents. These studies should also increase our understanding of "folding cores" and aid in biomaterial design and redesign. These studies have paved the way for our current more applied studies of biomaterial design, using the hydrophobic effect as a driving force that can be modulated in a variety of ways.

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